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(19)(CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) N-Phenylthiourea Derivatives and Pharmaceutical Use Thereof

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(73) Same as inventor

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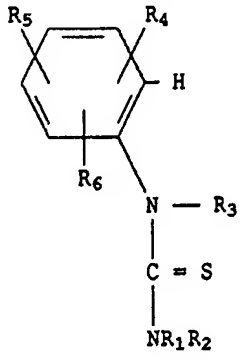
A B S T R A C T:

N-PHENYLTHIOUREA DERIVATIVES AND PHARMACEUTICAL USE THEREOF

The invention concerns the use of the compounds of formula I



I

wherein the substituents have various significances, in free form or salt form as appropriate, in the treatment of atherosclerosis.

It also concerns pharmaceutical compositions comprising a compound selected from a subgroup thereof. It further concerns a subgroup of compounds thereof, which are novel, per se.

The compounds of formula I can be prepared in conventional manner, e.g. by reaction of an isothiocyanate with an amine or conversion of an urea to a thiourea.

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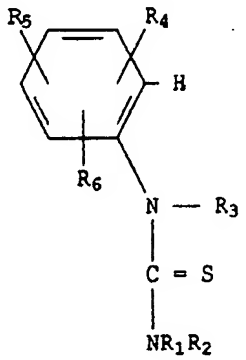
N-PHENYLTHIOUREA DERIVATIVES AND PHARMACEUTICAL USE THEREOF

The invention relates to N-phenylthiourea derivatives and their pharmaceutical use.

It concerns the use of a compound of formula I



I

wherein

either

R_1 is hydrogen; alkyl of 1 to 22 carbon atoms; cycloalkyl of 3 to 6 carbon atoms optionally mono- or independently disubstituted by alkyl of 1 or 2 carbon atoms; cycloalkylalkyl of 3 to 6 carbon atoms in the cycloalkyl and of 1 to 3 carbon atoms in the alkylene part or phenylalkyl of altogether 7 to 9 carbon atoms; hydroxyalkyl, alkoxyalkyl or mercaptoalkyl of 2 to 4 carbon atoms in the alkylene part, of 1 to 3 carbon atoms in the alkoxy part, and with the hydroxy, alkoxy or mercapto part attached in other than the 1-position; $-CR_7R_8R_9$ or $-CH_2CR_7R_8R_9$ wherein R_7 and R_8 either independently are hydrogen, methyl or ethyl or together are unbranched alkylene of 3 to 5 carbon atoms, and R_9 is carboxy, alkoxycarbonyl of altogether 2 to 4 carbon atoms or carbamoyl; alkenyl of 3 to 22 carbon atoms wherein the double bond is in other than the 1-position, or alkadienyl of 5 to 22 carbon atoms or alkatrienyl of 7 to 22 carbon atoms wherein each double bond is in other than the 1-position and no carbon atom is part of two double bonds, and

R_2 is hydrogen;

or

$-NR_1R_2$ is pyrrolidino, piperidino, hexamethyleneimino or morpholino, optionally mono-, di- or trisubstituted by alkyl of 1 to 4 carbon atoms or monosubstituted by hydroxymethyl or dialkylaminomethyl of independently 1 to 3 carbon atoms in the alkyl parts; piperazino substituted at the second nitrogen atom by alkyl of 1 to 4 carbon atoms, cycloalkyl of 5 or 6 carbon atoms, phenylalkyl of altogether 7 to 9 carbon atoms, phenyl, formyl or alkylcarbonyl of altogether 2 to 4 carbon atoms; 3-azaspiro[5.5]undec-3-yl; or 3,4-benzopiperidino;

R_3 is hydrogen or alkyl of 1 to 3 carbon atoms;

R_4 is hydrogen; alkyl of 1 to 4 carbon atoms; alkoxy of 1 to 3 carbon atoms; halogen of atomic number of from 9 to 35; trifluoromethyl; cyano; dialkylamino of independently 1 to 3 carbon atoms in the alkyl parts; nitro; phenyl; or benzyl;

R_5 is hydrogen; alkyl of 1 to 4 carbon atoms; alkoxy of 1 to 3 carbon atoms; halogen of atomic number 9 or 17; or trifluoromethyl; and

R_6 is hydrogen; alkyl of 1 to 3 carbon atoms; or alkoxy of 1 to 3 carbon atoms;

with the provisos that

i) when both R_4 and R_5 are trifluoromethyl,
then they are not ortho to each other;

ii) when R_1 is hydroxyalkyl and each of R_4, R_5 and R_6 is selected from hydrogen and alkyl,
then at least two of R_4, R_5 and R_6 are alkyl;

iii) when R_1 is alkyl and R_4 is phenyl,
then R_5 and R_6 are other than alkoxy; and

iv) when $-NR_1R_2$ is N'-alkylpiperazino and one of R_4 and R_5 is chloro,
then both the other of R_4 and R_5, and R_6, are other than alkoxy;

in free form or salt form as appropriate,

in the prophylactic or curative treatment of atherosclerosis.

As appears from the formula a compound of formula I cannot be substituted in both positions on the phenyl ring ortho to the nitrogen moiety.

A compound of formula I may be in free form, e.g. as a base, or salt form, e.g. anionic or acid addition salt form where such forms exist, as appropriate. Preferred acid addition salt forms are pharmaceutically acceptable acid addition salt forms, preferred anionic salt forms are salts with pharmaceutically acceptable cations. Included are salts with organic acids, e.g. the methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, acetate and propionate salts, and salts with inorganic acids, e.g. the hydrochloride and sulfate salts.

R_1 preferably is alkyl, cycloalkyl, cycloalkylalkyl, alkoxyalkyl, alkenyl, or a group $-CR_7R_8R_9$ or $-CH_2CR_7R_8R_9$ as defined above. R_2 and R_3 preferably are hydrogen. R_4 preferably is halogen, alkyl or benzyl, especially halogen or alkyl, particularly halogen. It preferably is in the

5-position. R_5 preferably is hydrogen, alkyl or alkoxy, especially alkyl. It preferably is in the 2-position. R_6 preferably is hydrogen or alkoxy, especially hydrogen. When it is other than hydrogen it preferably is in the 4-position. R_7 and R_8 preferably are methyl or ethyl, especially methyl. R_9 preferably is alkoxycarbonyl or carbamoyl.

Some of the compounds of formula I have one or more centers of asymmetry. Therefore, there are two or more enantiomers of each such compound. All of the possible isomers and racemates are within the scope of the invention. The compounds preferably are free of centers of asymmetry.

Alkyl of 1 to 22 carbon atoms preferably is of 1 to 8, especially of 3 to 5, particularly of 3 or 4 carbon atoms. When it is of more than 3 carbon atoms it preferably is branched, preferably at a position other than at the carbon atom attached to the nitrogen atom. It preferably is propyl or isopropyl, or of 4 to 22 carbon atoms and branched at a position other than at the carbon atom attached to the nitrogen atom. It especially is propyl, isopropyl or 2-methylpropyl. It particularly is 2-methylpropyl.

Cycloalkyl preferably is of 3 to 5, especially of 3 or 5 carbon atoms. It preferably is unsubstituted. Cycloalkylalkyl preferably is of 1 carbon atom in the alkylene part and preferably of 3 to 5, especially 3 carbon atoms in the cycloalkyl part. Phenylalkyl preferably is benzyl. The alkylene part of hydroxyalkyl, alkoxyalkyl or mercaptoalkyl preferably is of 2 or 3 carbon atoms; it preferably is unbranched.

Alkoxy as a substituent or part of a substituent preferably is methoxy. Unbranched alkylene conveniently is of 3 carbon atoms. Alkoxycarbonyl preferably is methoxycarbonyl. Alkenyl preferably is of 3 to 18 carbon atoms. Alkadienyl and alkatrienyl preferably are of 7 to 18 carbon atoms.

Alkyl of 1 to 4 or of 1 to 3 carbon atoms preferably is methyl or ethyl, especially methyl. The alkyl parts of dialkylamino or dialkylaminomethyl preferably are methyl. Alkylcarbonyl preferably is acetyl. Halogen preferably is chlorine.

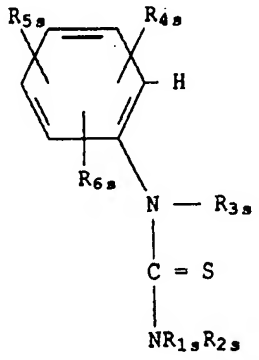
In a subgroup of compounds of formula I (**compounds Ip**) R_4 is other than benzyl and R_5 is other than alkyl of 4 carbon atoms.

In a further subgroup of compounds of formula I R_1 is other than phenylalkyl. In a further subgroup R_9 is other than carboxy. In a further subgroup R_4, R_5 and R_6 are other than halogen in ortho position to the nitrogen moiety. In a further subgroup R_4, R_5, and R_6 are other than alkyl of more than 1 carbon atom. In a further subgroup R_1 is other than hydrogen. In a further subgroup R_3 is hydrogen. In a further subgroup R_1 and R_2 do not form a cyclic structure together with the nitrogen atom. In a further subgroup, R_1 is propyl, isopropyl or 2-methylpropyl, R_2, R_3 and R_6 are hydrogen, R_4 is 5-halo and R_5 is 2-alkyl. In a further subgroup R_1 is alkyl of 8 to 22 carbon atoms.

Further subgroups of compounds of formula I are the compounds wherein

- R_1 is optionally substituted cycloalkyl as defined above;
- R_1 is cycloalkylalkyl or phenylalkyl as defined above;
- R_1 is $-CR_7R_8R_9$ or $-CH_2CR_7R_8R_9$ as defined above;
- R_1 is alkenyl as defined above, preferably of 5 to 22 carbon atoms;
- R_1 is alkadienyl or alkatrienyl as defined above;
- $-NR_1R_2$ is a cyclic moiety as defined above; and/or
- R_3 is alkyl of 1 to 3 carbon atoms.

A further subgroup of compounds of formula I is the compounds of formula Is



Is

wherein

either

R_{1s} is hydrogen; alkyl of 1 to 6 carbon atoms; cycloalkyl of 3 to 6 carbon atoms optionally monosubstituted by methyl; cyclopropylmethyl; phenylalkyl of 7 or 8 carbon atoms; hydroxyalkyl of 2 to 4 carbon atoms, methoxyethyl or mercaptoethyl with the hydroxy, methoxy or mercapto part attached in other than the 1-position; $-CR_{7s}R_{8s}R_{9s}$ or $-CH_2CR_{7s}R_{8s}R_{9s}$ wherein R_{7s} and R_{8s} either independently are hydrogen or methyl or together are unbranched alkylene of 3 to 5 carbon atoms, and R_{9s} is carboxy, methoxycarbonyl or carbamoyl; alkenyl of 3 to 18 carbon atoms wherein the double bond is in other than the 1-position, or alkadienyl of 5 to 18 carbon atoms or alkatrienyl of 7 to 18 carbon atoms wherein each double bond is in other than the 1-position and no carbon atom is part of two double bonds, and

R_{2s} is hydrogen;

or

$-NR_{1s}R_{2s}$ is pyrrolidino, piperidino, hexamethyleneimino or morpholino optionally mono- or disubstituted by methyl or ethyl or monosubstituted by hydroxymethyl or dimethylaminomethyl; piperazino substituted at the second nitrogen atom by alkyl of 1 to 3 carbon atoms, cyclohexyl, benzyl, 2-phenylethyl, phenyl, formyl or acetyl; 3-azaspiro[5.5]undec-3-yl; or 3,4-benzopiperidino;

R_{3s} is hydrogen or methyl;

R_{4s} is hydrogen; alkyl of 1 to 4 carbon atoms; methoxy; halogen of atomic number of from 9 to 35; trifluoromethyl; cyano; dimethylamino; nitro; phenyl; or benzyl;

R_{5s} is hydrogen; alkyl of 1 to 4 carbon atoms; methoxy; halogen of atomic number 9 or 17; or trifluoromethyl; and

R_{6s} is hydrogen; alkyl of 1 to 3 carbon atoms; or methoxy;

with the provisos that

i) when both R_{4s} and R_{5s} are trifluoromethyl,
then they are not ortho to each other;

ii) when R_{1s} is hydroxyalkyl and each of R_{4s} and R_{5s} is selected from hydrogen and alkyl,
then at least two of R_{4s}, R_{5s} and R_{6s} are alkyl;

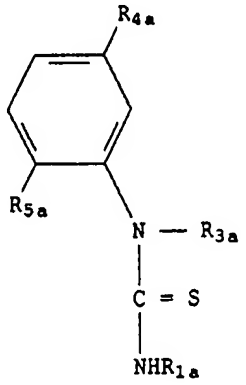
iii) when R_{1s} is alkyl and R_{4s} is phenyl,
then R_{5s} and R_{6s} are other than methoxy; and

iv) when $-NR_{1s}R_{2s}$ is N'-alkylpiperazino and one of R_{4s} and R_{5s} is chloro,
then both the other of R_{4s} and R_{5s}, and R_{6s}, are other than methoxy;

in free form or salt form as appropriate.

While many of the compounds of formula I are known per se, as well as their use in therapy, such as the compound of Example 99, from e.g. Rohm and Haas USP 3'950'537 as compound M therein, their use in the therapy of atherosclerosis is novel.

Further, for a subgroup of compounds of formula I no therapeutic use at all is known. The invention therefore also concerns a pharmaceutical composition comprising a compound of formula Ia



Ia

wherein

R_{1a} is hydrogen; alkyl of 3 to 22 carbon atoms; cycloalkyl of 3 to 6 carbon atoms optionally mono- or independently disubstituted by alkyl of 1 or

2 carbon atoms; cycloalkylalkyl of 3 to 6 carbon atoms in the cycloalkyl and of 1 to 3 carbon atoms in the alkylene part; alkoxyalkyl or mercaptoalkyl of 2 to 4 carbon atoms in the alkylene part, of 1 to 3 carbon atoms in the alkoxy part, and with the alkoxy or mercapto part attached in other than the 1-position; $-CR_{7a}R_{8a}R_{9a}$ or $-CH_2CR_{7a}R_{8a}R_{9a}$ wherein R_{7a} and R_{8a} either independently are hydrogen, methyl or ethyl or together are unbranched alkylene of 3 to 5 carbon atoms, and R_{9a} is alkoxycarbonyl of altogether 2 to 4 carbon atoms or carbamoyl; alkenyl of 4 to 22 carbon atoms wherein the double bond is in other than the 1-position, or alkadienyl of 5 to 22 carbon atoms or alkatrienyl of 7 to 22 carbon atoms wherein each double bond is in other than the 1-position and no carbon atom is part of two double bonds;

R_{3a} is hydrogen or alkyl of 1 to 3 carbon atoms;

one of R_{4a} and R_{5a} is alkyl of 1 to 4 carbon atoms; alkoxy of 1 to 3 carbon atoms; halogen of atomic number of from 9 to 35; trifluoromethyl; cyano; dialkylamino of independently 1 to 3 carbon atoms in the alkyl parts; nitro; phenyl; or benzyl;

the other of R_{4a} and R_{5a} is alkyl of 1 to 4 carbon atoms; alkoxy of 1 to 3 carbon atoms; or trifluoromethyl;

with the provisos that

i) when both R_{4a} and R_{5a} are trifluoromethyl, then they are not ortho to each other; and

ii) when R_{1a} is alkyl and one of R_{4a} and R_{5a} is phenyl, then the other of R_{4a} and R_{5a} is other than alkoxy;

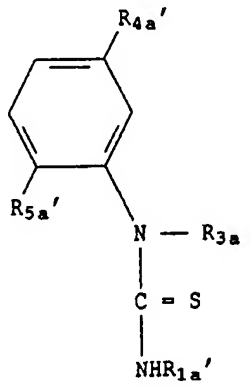
in free form or salt form as appropriate,

together with a pharmaceutically acceptable carrier or diluent.

In a subgroup of compounds of formula Ia R_{1a} is other than hydrogen. In a further subgroup R_{4a} and R_{5a} are other than both alkoxy. In a further subgroup R_{1a} when it is branched alkyl of more than 3 carbon atoms is branched at a position other than at the carbon atom attached to the nitrogen atom. In a further subgroup R_{1a} when it is alkyl is propyl or isopropyl or alkyl of 4 to 22 carbon atoms branched at a position other than at the carbon atom attached to the nitrogen atom. In a further

subgroup R_{1a} when it is alkyl is propyl, isopropyl or 2-methylpropyl. In a further subgroup R_{4a} and R_{5a} when they are alkyl are methyl; in a further subgroup they are other than benzyl.

A preferred group of compounds of formula Ia is the compounds of formula Ia'



Ia'

wherein

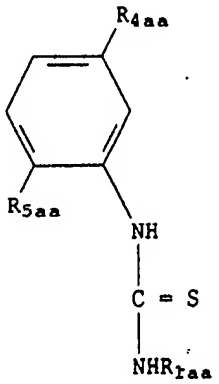
R_{1a}' is propyl or isopropyl or alkyl of 4 to 22 carbon atoms branched at a position other than at the carbon atom attached to the nitrogen atom; cycloalkyl of 3 to 6 carbon atoms optionally mono- or independently disubstituted by alkyl of 1 or 2 carbon atoms; cycloalkylalkyl of 3 to 6 carbon atoms in the cycloalkyl and of 1 to 3 carbon atoms in the alkylene part; alkoxyalkyl or mercaptoalkyl of 2 to 4 carbon atoms in the alkylene part, of 1 to 3 carbon atoms in the alkoxy part, and with the alkoxy or mercapto part attached in other than the 1-position; $-CR_{7a}R_{8a}R_{9a}$ or $-CH_2CR_{7a}R_{8a}R_{9a}$ wherein R_{7a}, R_{8a} and R_{9a} are as defined above; alkenyl of 4 to 22 carbon atoms wherein the double bond is in other than the 1-position, or alkadienyl of 5 to 22 carbon atoms or alkatrienyl of 7 to 22 carbon atoms wherein each double bond is in other than the 1-position and no carbon atom is part of two double bonds;

R_{3a} is as defined above;
one of R_{4a}' and R_{5a}' is methyl; alkoxy of 1 to 3 carbon atoms; halogen of atomic number of from 9 to 35; trifluoromethyl; cyano; dialkylamino of independently 1 to 3 carbon atoms in the alkyl parts; nitro; phenyl; or benzyl;
the other of R_{4a}' and R_{5a}' is methyl; alkoxy of 1 to 3 carbon atoms; or trifluoromethyl;
with the provisos that
i) when both R_{4a}' and R_{5a}' are trifluoromethyl, then they are not ortho to each other;
ii) when R_{1a}' is alkyl and one of R_{4a}' and R_{5a}' is phenyl, then the other of R_{4a}' and R_{5a}' is other than alkoxy; and
iii) R_{4a}' and R_{5a}' are other than both alkoxy,
in free form or salt form as appropriate,
together with a pharmaceutically acceptable carrier or diluent.

In a subgroup of compounds of formula Ia' R_{1a}' is propyl, isopropyl or 2-methylpropyl.

While some of the compounds of formula Ia are known per se, such as the compound of Example 29, from e.g. Hoechst USP 4'234'513 as Example 27 therein, and the compound of Example 30, from e.g. G.N. Vassilev and L.K. Iliev, Comptes Rendus Acad. bulg. Sci. 22 (5) (1969) 563-566 as compound 10. therein, their therapeutic use is novel.

Further, a particular subgroup of compounds of formula Ia is novel per se. The invention thus also concerns a compound of formula Iaa



Iaa

wherein

R_{1aa} is propyl, isopropyl or 2-methylpropyl;

R_{4aa} is halogen of atomic number of from 9 to 35; and

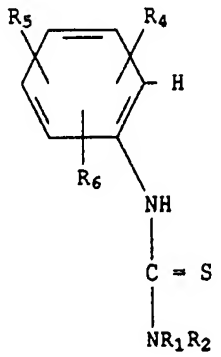
R_{5aa} is alkyl of 1 to 4 carbon atoms.

R_{1aa} preferably is propyl or 2-methylpropyl. R_{4aa} preferably is chlorine. R_{5aa} preferably is methyl, ethyl, isopropyl or t-butyl, preferably methyl, ethyl or isopropyl, especially methyl.

In a subgroup of compounds of formula Iaa R_{1aa} is propyl or 2-methylpropyl and R_{5aa} is alkyl of 1 to 3 carbon atoms; more particularly, R_{1aa} is propyl, R_{4aa} if fluoro or chloro and R_{5aa} is methyl or ethyl; even more particularly, R_{5aa} is methyl; even more particularly, R_{1aa} is 2-methylpropyl.

A part of the compounds of formula I is known and those which are not may be synthesized analogously to the known compounds of formula I. For example, a compound of formula I can be obtained by a process which comprises

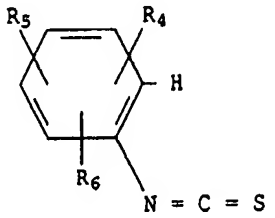
a) for the preparation of a compound of formula I'



I'

wherein the substituents are as defined above,

reacting a corresponding compound of formula II



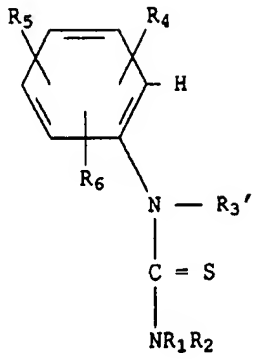
II

with a corresponding compound of formula III

R_1R_2NH III

or

b) for the preparation of a compound of formula I"

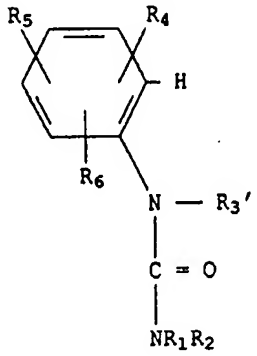


I"

wherein R_3' is alkyl of 1 to 3 carbon atoms and

the remaining substituents are as defined above,

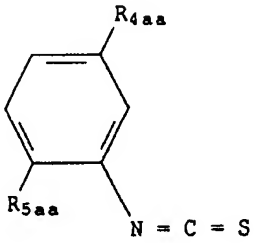
appropriately converting the oxo moiety to a thiono moiety in a corresponding compound of formula IV



IV

and recovering the resultant compound of formula I in free form or salt form as appropriate.

Thus a compound of formula Iaa can be obtained by a process which comprises reacting a corresponding compound of formula IIa



IIa

wherein R_{4aa} and R_{5aa} are as defined above,
with a corresponding compound of formula IIIa

$$R_{1aa}NH_2 \qquad \text{IIIa}$$

wherein R_{1aa} is as defined above.

The above process for the preparation of the compounds of formula I can be effected in conventional manner.

Process variant a) is a reaction of an isothiocyanate with an amine. The temperature preferably is from about 10° to about 40°C, preferably about 20° to about 30°C. An anhydrous inert organic solvent conveniently is used, such as a halogenated lower alkane, e.g. methylene chloride, or a C_{1-3}alkyl (C_{2-3})alkanoate, e.g. ethyl acetate.

Process variant b) is a conversion of an urea to the corresponding thiourea. It preferably is effected with Lawessons's reagent (2,4-bis-4-methoxyphenyl-1,3-dithia-2,4-diphosphethane-2,4-disulfide). Preferably from about 0.5 to about 1 mole, especially from about 0.6 to about 0.85 mole Lawesson's reagent is used per mole compound of formula VI. The temperature preferably is from about 50°C to reflux temperature, preferably from about 100° to about 110°C, especially about 110°C. An anhydrous inert organic solvent conveniently is used, preferably a hydrocarbon such as benzene, toluene or xylene, especially toluene.

A compound of formula I can be isolated and, if desired, purified from the reaction mixture in conventional manner.

A compound in free form can be converted to a salt form where appropriate also in conventional manner, and vice-versa.

A compound having one or more centers of asymmetry may be obtained in optically pure form by using an optically pure starting material, or may be resolved into two optically active isomers by conventional techniques, e.g. via formation of a mixture of diastereoisomeric salts where salt formation is possible and subsequent separation by e.g. fractional crystallization or column chromatography.

Insofar as its preparation is not described herein, a compound used as a starting material is known or can be prepared by known methods starting from known compounds, e.g. as described in the Examples.

The following Examples illustrate the invention. All temperatures are in degrees Centigrade. MP = melting point; OR = optical rotation $[\alpha]_D^{20}$. The NMR spectra are taken at 200 MHz.

Example 1: N-(5-Chloro-2-methylphenyl)-N'-propylthiourea

[Formula I: R_1 = n-propyl; R_2, R_3, R_6 = H; R_4 = 5-chloro; R_5 = 2-methyl]
[Formula Iaa: R_{1aa} = n-propyl; R_{4aa} = chloro; R_{5aa} = methyl]

[Process variant a); reaction of an isothiocyanate with an amine]

163 g **n-propylamine** (compound of formula III) is added dropwise over a period of 40 minutes to a solution of 390 g of **5-chloro-2-methylphenylisothiocyanate** (compound of formula II) in 700 ml of ethyl acetate stirred at 15° under nitrogen at a rate such that the temperature is maintained at 25°-30°. On completion of the addition, the reaction mixture is stirred at 25°-30° for 5 minutes, 2.1 l of n-heptane is added, and the mixture is cooled to 0° over a period of 20 minutes. The resulting white suspension is stirred at 0° for 1 hour, and the solids are collected by vacuum filtration, washed with two 250 ml portions of cold (5°) n-heptane and dried at 25 torr and 40° for about 18 hours to constant weight. The **title compound** is obtained (white solid; MP 97°-98°).

Example 2: N-(5-Chloro-2-methylphenyl)-N'-2-methylpropylthiourea

[Formula I: R_1 = 2-methylpropyl; R_2, R_3, R_6 = H; R_4 = 5-chloro; R_5 = 2-methyl]
[Formula Iaa: R_{1aa} = 2-methylpropyl; R_{4aa} = chloro; R_{5aa} = methyl]

[Process variant a)]

A solution of 25.0 g of **5-chloro-2-methylphenylisothiocyanate** (compound of formula II) in 25 ml of methylene chloride is added dropwise to a solution of 10.0 g of **isobutylamine** (compound of formula III) in 150 ml of methylene chloride stirred at 20°-25°, and the reaction mixture is stirred at 20°-25° for 2 hours. The methylene chloride is distilled, and, as the distillation proceeds, methyl-t-butyl ether is gradually added.

The resulting methyl t-butyl ether solution is allowed to cool to 20°-25°, and the resulting solid is collected by filtration, washed with methyl t-butyl ether and vacuum dried to constant weight. The title compound is obtained (MP 116.5°-117.5°)

Example 3: N-(5-Chloro-2-methylphenyl)-N-methyl-N'-propylthiourea

[Formula I: R_1 = n-propyl; R_2, R_6 = H; R_3 = methyl; R_4 = 5-chloro; R_5 = 2-methyl]
[Formula Ia: R_{1a} = n-propyl; R_{3a}, R_{5a} = methyl; R_{4a} = chloro]

[Process variant b); conversion]

A mixture of 1.8 g of crude **N-(5-chloro-2-methylphenyl)-N-methyl-N'-propylurea** (compound of formula IV) prepared as described hereunder and 2.5 g of Lawesson's reagent in 20 ml of toluene is stirred at 110° for 24 hours, the toluene is evaporated at reduced pressure, and the waxy solid residue is dissolved in the minimum amount of methylene chloride and flash chromatographed on a 230-400 mesh ASTM silica gel column. The fractions containing relatively pure product as determined by thin layer chromatography are combined and evaporated at reduced pressure to obtain an oil which crystallizes upon standing. The crystals are triturated with n-pentane to obtain the **title compound** (MP 83°-86°). A less pure second crop is similarly obtained from less pure fractions from the flash chromatography (MP 82°-84°).

The starting material is obtained as follows:

a) 1.5 g of **n-propylamine** is slowly added to a solution of 4.3 g of **5-chloro-2-methylphenylisocyanate** in 70 ml of methylene chloride stirred at 20°-25°, and, after the vigorous reaction subsides, the reaction mixture is stirred at 20°-25° for 10 minutes. The resulting solid is collected by filtration and washed with methylene chloride. **N-(5-chloro-2-methylphenyl)-N'-propylurea** is obtained (MP 182°-184°).

b) 600 mg of 60 % sodium hydride/mineral oil is washed with n-pentane, the sodium hydride is added to a solution of 2.7 g of **product from step a) above** in 25 ml of N,N-dimethylformamide stirred at 20°-25°, the mixture is stirred at 20°-25° for 1.5 hours, 1.8 g of **methyl iodide** is slowly added, and the reaction mixture is stirred at 20°-25° for 24 hours and poured into water. The mixture is extracted twice with methyl t-butyl ether, and the ether extracts are combined, dried over anhydrous sodium sulfate and evaporated at reduced pressure to an oil. The oil is fractionated on a Waters HPLC having a silica gel column using 60 % mixed hexanes/ethyl acetate as the eluant. **N-(5-chloro-2-methylphenyl)-N-methyl-N'-propylurea** is obtained (waxy solid).

The following compounds of formula I (and Ia and Iaa) are obtained in analogous manner:

Example No.	Process variant	Analogously to Ex. No.	R_1	R_2	R_3	R_4	R_5	R_6	MP and/or OR
4	a)	1; 2	$-CH(CH_3)_2$	H	H	5-Cl	$2-CH_3$	H	126°-128°
5	a)	1; 2	$-CH_2CH_2CH_3$	H	H	5-Cl	$2-CH_2CH_3$	H	79°- 81°
6	a)	1; 2	$-CH_2CH_2CH_3$	H	H	5-F	$2-CH_3$	H	68°- 71°
7	a)	1; 2	$-CH_2CH_2CH_3$	H	H	5-Br	$2-CH_3$	H	109°-112°

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The following compounds of formula I (and Ia) are obtained in analogous manner:

Example No.	Process variant	Analogously to Ex. No.	R_1	R_2	R_3	R_4	R_5	R_6	MP and/or OR
8	a)	1; 2	$-CH_2CH_2OCH_3$	H	H	5-Cl	2-CH_3	H	149°-150°
9	a)	1; 2	(Z)-$-(CH_2)_8CH=CH(CH_2)_7CH_3$	H	H	5-Cl	2-CH_3	H	waxy semi-solid; NMR[1)]
10	a)	1; 2	$-CH_2CH_2CH_2CH_2CH_3$	H	H	5-Cl	2-CH_3	H	80°- 82°
11	a)	1; 2	cyclopropylmethyl	H	H	5-Cl	2-CH_3	H	146°-147°
12	a)	1; 2	$-CH_2CH_2CH_3$	H	H	5-Cl	2-OCH_3	H	151°-154°
13	a)	1; 2	cyclohexyl	H	H	5-Cl	2-CH_3	H	156°-158°
14	a)	1; 2	cyclopentyl	H	H	5-Cl	2-CH_3	H	161°-163°
15	a)	1; 2	cyclobutyl	H	H	5-Cl	2-CH_3	H	136°-138°
16	a)	1; 2	cyclopropyl	H	H	5-Cl	2-CH_3	H	204°-207°
17	a)	1; 2	$-CH_2C(CH_3)_2CONH_2$	H	H	5-Cl	2-CH_3	H	130°-132°
18	a)	1; 2	$-CH_2CH_2CH_3$	H	H	5-CH_3	2-CH_3	H	65°- 68°
19	a)	1; 2	$-CH_2CH_2CH_3$	H	H	5-OCH_3	2-OCH_3	H	103°-106°
20	a)	1; 2	$-C(CH_3)_3$	H	H	5-Cl	2-CH_3	H	135°-138°
21	a)	1; 2	$-C(CH_3)_2CH_2CH_3$	H	H	5-Cl	2-CH_3	H	106°-109°
22	a)	1; 2	(E,E)-$[CH_2CH=C(CH_3)CH_2-]_2-CH_2CH=C(CH_3)_2$	H	H	5-Cl	2-CH_3	H	40°- 42°
23	a)	1; 2	$-CH_2CH_2CH_3$	H	H	5-CH_3	2-OCH_3	H	110°-113°
24	a)	1; 2	(RS)-$CH(CH_3)C(CH_3)_3$	H	H	5-Cl	2-CH_3	H	135°-138°
25	a)	1; 2	$-CH_2CH_2SH$	H	H	5-Cl	2-CH_3	H	107°-109°
26	a)	1; 2	(RS)-2-methylcyclohexyl	H	H	5-Cl	2-CH_3	H	164°-170°
27	a)	1; 2	$-CH_2C(CH_3)_2COOCH_3$	H	H	5-Cl	2-CH_3	H	94°- 96°
28	a)	1; 2	$-CH_2C(CH_3)_3$	H	H	5-Cl	2-CH_3	H	126°-130°

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The following further compounds of formula I (and Ia) are obtained in analogous manner:

Example No.	Process variant	Analogously to Ex. No.	R_1	R_2	R_3	R_4	R_5	R_6	MP and/or OR
29	a)	1; 2	H	H	H	5-Cl	2-CH_3	H	140°-143°
30	a)	1; 2	$-CH_2CH_2CH_2CH_3$	H	H	5-Cl	2-CH_3	H	108°-110°

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The following compounds of formula I are obtained in analogous manner:

Example No.	Process variant	Analogously to Ex. No.	R_1	R_2	R_3	R_4	R_5	R_6	MP and/or OR
31	a)	1; 2	$-C(CH_3)_2CH_2OH$	H	H	5-Cl	2-CH_3	H	120°-122°
32	a)	1; 2	$-(CH_2)_3OH$	H	H	5-Cl	2-CH_3	H	103°-107°
33	a)	1; 2	$-CH_2CH_2COOH$	H	H	5-Cl	2-CH_3	H	180°-183°
34	a)	1; 2	$-CH_2CH_2OH$	H	H	5-Cl	2-OCH_3	H	105°-108°
35	a)	1; 2	$-CH_2CH_2OH$	H	H	3-Cl	4-CH_3	H	148°-151°
36	a)	1; 2	(S)$-CH(CH_3)CH_2OH$	H	H	5-Cl	2-CH_3	H	127°-130° -35.1° (c=1.02,CH_3OH)
37	a)	1; 2	(R)$-CH(CH_3)CH_2OH$	H	H	5-Cl	2-CH_3	H	132°-134° +34.6° (c=1.03,CH_3OH)
38	a)	1; 2	(R)$-CH_2CH(CH_3)OH$	H	H	5-Cl	2-CH_3	H	92°- 95° -11.8° (c=1.01,$CHCl_3$)
39	a)	1; 2	(S)$-CH_2CH(CH_3)OH$	H	H	5-Cl	2-CH_3	H	88°- 92° +10.0° (c=1.00,$CHCl_3$)
40	a)	1; 2	$-CH_2CH_2OH$	H	H	5-Cl	3-Cl	H	148°-151°
41	a)	1; 2	$-CH_2C(CH_3)_2COOH$	H	H	5-Cl	2-CH_3	H	168°-170°
42	a)	1; 2	$-CH_2C(CH_3)_2COOH$	H	H	5-CH_3	2-CH_3	H	174°-176°
43	a)	1; 2	$-CH_2C(CH_3)_2COOH$	H	H	5-Cl	2-OCH_3	H	138°-141°
44	a)	1; 2	(1-carboxycyclobutyl)methyl	H	H	5-Cl	2-CH_3	H	156°-159°
45	a)	1; 2	(1-carboxycyclopentyl)methyl	H	H	5-Cl	2-CH_3	H	151°-154°
46	a)	1; 2	(1-carboxycyclohexyl)methyl	H	H	5-Cl	2-CH_3	H	150°-152°
47	a)	1; 2	$-CH_2CH_2CH_3$	H	H	5-CH_3	3-CH_3	H	104°-107°
48	a)	1; 2	$-CH_2CH_2CH_3$	H	H	3-Cl	2-OCH_3	H	93°- 96°

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Example No.	Process variant	Analogously to Ex. No.	R_1	R_2	R_3	R_4	R_5	R_6	MP and/or OR
49	a)	1; 2	benzyl	H	H	5-Cl	2-CH_3	H	160°-162°
50	a)	1; 2	2-phenylethyl	H	H	5-Cl	2-CH_3	H	124°-127°
51	a)	1; 2	$-CH_2CH_2CH_3$	H	H	5-Cl	2-OCH_3	4-OCH_3	141°-142°
52	a)	1; 2	$-CH_2COOH$	H	H	5-Cl	2-CH_3	H	169°-170°
53	a)	1; 2	$-CH_2CH_2OH$	H	H	5-Cl	2-Cl	H	125°-128°
54	a)	1; 2	$-CH_2C(CH_3)_2CH_2CH_2CH_2-$		H	5-Cl	2-CH_3	H	110°-113°
55	a)	1; 2	$-CH_2CH_2OCH_2CH_2-$		H	5-Cl	2-CH_3	H	162°-164°
56	a)	1; 2	$-(CH_2)_5-$		H	5-Cl	2-CH_3	H	103°-106°
57	a)	1; 2	$-CH_2CH_2N(CH_3)CH_2CH_2-$		H	5-Cl	2-CH_3	H	122°-124°
58	a)	1; 2	$-(RS)-CH(CH_2OH)CH_2CH_2CH_2CH_2-$		H	5-Cl	2-CH_3	H	132°-135°
59	a)	1; 2	$-(R)-CH(CH_2OH)CH_2CH_2CH_2CH_2-$		H	5-Cl	2-CH_3	H	+ 214.5° (c=1.6, $CHCl_3$)
60	a)	1; 2	$-(S)-CH(CH_2OH)CH_2CH_2CH_2CH_2-$		H	5-Cl	2-CH_3	H	- 251.3° (c=0.99,$CHCl_3$)
61	a)	1; 2	$-(CH_2)_4-$		H	5-Cl	2-CH_3	H	149°-151°
62	a)	1; 2	$-CH_2CH_2N(C_6H_5)CH_2CH_2-$		H	5-Cl	2-CH_3	H	179°-181°
63	a)	1; 2	$-CH_2CH_2N(CHO)CH_2CH_2-$		H	5-Cl	2-CH_3	H	118°-121°
64	a)	1; 2	3,4-benzopiperidino (together with N atom)		H	5-Cl	2-CH_3	H	158°-160°
65	a)	1; 2	$-(RS)-CH(CH_2CH_3)CH_2CH_2CH_2CH_2-$		H	5-Cl	2-CH_3	H	102°-105°
66	a)	1; 2	$-(R)-CH(CH_2CH_3)CH_2CH_2CH_2CH_2-$		H	5-Cl	2-CH_3	H	124°-126° +32.4° (c=1.09,C_2H_5OH)
67	a)	1; 2	$-(S)-CH(CH_2CH_3)CH_2CH_2CH_2CH_2-$		H	5-Cl	2-CH_3	H	122°-124° -32.7° (c=1.00,C_2H_5OH)
68	a)	1; 2	$-CH_2CH_2N(CH_2C_6H_5)CH_2CH_2-$		H	5-Cl	2-CH_3	H	120°-123°

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Example No.	Process variant	Analogously to Ex. No.	R_1	R_2	R_3	R_4	R_5	R_6	MP and/or OR
69	a)	1; 2	$-CH_2CH_2N(CH_3)CH_2CH_2-$		H	3-CF_3	H	H	149°-150°
70	a)	1; 2	$-CH_2CH_2N(CH_3)CH_2CH_2-$		H	2-CF_3	H	H	111°-114°
71	a)	1; 2	$-CH_2CH_2N(CH_3)CH_2CH_2-$		H	4-CF_3	H	H	172°-174°
72	a)	1; 2	$-CH_2CH_2N(COCH_3)CH_2CH_2-$		H	5-Cl	2-CH_3	H	152°-155°
73	a)	1; 2	$-CH[CH_2N(CH_3)_2]CH_2CH_2CH_2CH_2-$		H	5-Cl	2-CH_3	H	114°-117°
74	a)	1; 2	$-CH_2CH_2N(CH_2CH_2C_6H_5)CH_2CH_2-$		H	5-Cl	2-CH_3	H	164°-166°
75	a)	1; 2	$-CH_2CH_2N[CH(CH_3)_2]CH_2CH_2-$		H	5-Cl	2-CH_3	H	165°-167°
76	a)	1; 2	$-CH_2CH_2N(cyclohexyl)CH_2CH_2-$		H	5-Cl	2-CH_3	H	152°-154°
77	a)	1; 2	$-(CH_2)_6-$		H	5-Cl	2-CH_3	H	142°-145°
78	a)	1; 2	$-CH_2CH_2CH(CH_3)CH_2CH_2-$		H	5-Cl	2-CH_3	H	145°-148°
79	a)	1; 2	$-CH_2CH_2OCH_2CH_2-$		H	5-Cl	2-OCH_3	H	140°-143°
80	a)	1; 2	$-CH_2CH_2N(CH_3)CH_2CH_2-$		H	4-$N(CH_3)_2$	H	H	141°-143°
81	a)	1; 2	$-CH_2CH_2N(CH_3)CH_2CH_2-$		H	5-F	2-CH_3	H	137°-139°
82	a)	1; 2	$-CH_2CH_2N(CH_3)CH_2CH_2-$		H	2-C_6H_5	H	H	157°-160°
83	a)	1; 2	$-CH_2CH_2N(CH_3)CH_2CH_2-$		H	5-NO_2	2-CH_3	H	157°-160°
84	a)	1; 2	$-CH_2CH_2N(CH_3)CH_2CH_2-$		H	5-C_6H_5	2-OCH_3	H	117°-120°
85	a)	1; 2	$-CH_2CH_2N(CH_3)CH_2CH_2-$		H	5-CH_3	3-CH_3	H	155°-157°
86	a)	1; 2	$-CH_2C(CH_3)_2CH_2CH_2CH_2-$		H	5-Cl	2-OCH_3	H	oil; NMR[2]
87	a)	1; 2	$-CH_2CH_2CH(CH_3)CH_2CH_2-$		H	5-Cl	2-OCH_3	H	68°- 71°
88	a)	1; 2	$-CH_2CH_2C[-(CH_2)_5-]CH_2CH_2-$		H	5-Cl	2-CH_3	H	186°-189°
89	a)	1; 2	$-CH_2CH_2C[(CH_3)_2]CH_2CH_2-$		H	5-Cl	2-CH_3	H	138°-141°
90	a)	1; 2	$-cis-CH(CH_3)CH_2CH_2CH_2CH(CH_3)-$		H	5-Cl	2-CH_3	H	112°-115°
91	a)	1; 2	$-(R)-CH(CH_2OH)CH_2CH_2CH_2-$		H	5-Cl	2-CH_3	H	132°-135° +109.1° (c=1.04,CH_3OH)
92	a)	1; 2	$-(S)-CH(CH_2OH)CH_2CH_2CH_2-$		H	5-Cl	2-CH_3	H	132°-135° -108.3° (c=1.09,CH_3OH)

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Example No.	Process variant	Analogously to Ex. No.	R_1	R_2	R_3	R_4	R_5	R_6	MP and/or OR
93	a)	1; 2	$-CH_2CH_2CH_3$	H	H	4-Cl	$2-CF_3$	H	126°-129°
94	a)	1; 2	$-CH_2CH(CH_3)CH_3$	H	H	4-Cl	$2-CH_3$	H	115°-118°
95	a)	1; 2	$-CH_2CH_2CH_3$	H	H	$2-CH_2C_6H_5$	H	H	95°- 98°
96	a)	1; 2	$-CH_2CH_2CH_3$	H	H	$2-CH(CH_3)_2$	H	H	68°- 74°
97	a)	1; 2	$-CH_2C_6H_5$	H	H	4-Cl	$2-CH_3$	H	125°-127°
98	a)	1; 2	$-CH_2CH(CH_3)CH_3$	H	H	$2-N(CH_3)_2$	H	H	110°-112°

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The following further compounds of formula I are obtained in analogous manner:

Example No.	Process variant	Analogously to Ex. No.	R_1	R_2	R_3	R_4	R_5	R_6	MP and/or OR
99	a)	1; 2	$-CH_2CH_2OH$	H	H	5-Cl	2-CH_3	H	134°-137°
100	a)	1; 2	$-CH_2CH_2OH$	H	H	3-Cl	H	H	135°-138°
101	a)	1; 2	$-CH_2CH_2OH$	H	H	3-Cl	2-CH_3	H	136°-139°
102	a)	1; 2	$-CH_2CH_2OH$	H	H	4-Cl	2-CH_3	H	121°-124°
103	a)	1; 2	$-CH_2CH_2OH$	H	H	5-CH_3	2-CH_3	H	157°-159°
104	a)	1; 2	$-CH_2CH_2OH$	H	H	3-CF_3	H	H	76°- 78°
105	a)	1; 2	$-CH_3$	H	H	5-Cl	2-CH_3	H	139°-142°
106	a)	1; 2	$-CH_2CH_3$	H	H	5-Cl	2-CH_3	H	116°-119°
107	a)	1; 2	$-CH_2CH=CH_2$	H	H	5-Cl	2-CH_3	H	94°- 96°
108	a)	1; 2	$-CH_2CH_2CH_3$	H	H	2-C_6H_5	H	H	114°-117°
109	a)	1; 2	(RS)-$CH(CH_3)C(CH_3)_3$	H	H	4-F	H	H	149°-151°
110	a)	1; 2	(RS)-$CH(CH_3)C(CH_3)_3$	H	H	4-CN	H	H	165°-168°
111	a)	1; 2	$-CH_2CH_2N(CH_3)CH_2CH_2-$		H	5-CF_3	3-CF_3	H	161°-163°
112	a)	1; 2	$-CH_2CH_2N(CH_3)CH_2CH_2-$		H	H	H	H	130°-133°
113	a)	1; 2	$-CH_2CH_2CH_3$	H	H	4-Cl	2-CH_3	H	64°- 67°
114	a)	1; 2	$-CH_2CH_2CH_3$	H	H	2-CH_3	H	H	58°- 63°
115	a)	1; 2	$-CH_2C_6H_5$	H	H	4-Cl	H	H	124°-125°

1) ^{1}H-NMR (CD_3SOCD_3): 0.84 (t,3H); 1.07-1.63 (m,22H); 1.84-2.09 (m,3H); 2.16 (s,3H); 3.23-3.52 (m,3H); 5.35 (t,2H); 7.12-7.45 (m,3H); 7.75 (s,1H); 9.02 (s,3H);

2) ^{1}H-NMR (CD_3SOCD_3): 0.84 (s,6H); 1.36 (m,2H); 1.48-1.60 (m,2H); 3.52 (s,2H); 3.63 (s,3H); 3.81 (m,2H); 6.96 (d,1H); 7.14-7.21 (m,2H); 8.53 (s,1H).

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The compounds of formula I in free form or pharmaceutically acceptable salt form as appropriate, possess interesting pharmacological activity. They are indicated for use as pharmaceuticals.

In particular, they raise the blood serum high density lipoprotein [HDL; HDL-cholesterol and apolipoprotein A-I (Apo A-I)] level. Most of the compounds have the added benefit of lowering the blood serum total triglyceride level.

This activity can be determined in conventional assay methods, e.g. as follows:

a) Test A: In vivo HDL-cholesterol test:

Male Sprague-Dawley rats weighing 200-225 g are housed two per cage and fed Purina Rodent Chow Special Mix 5001-S supplemented with 0.25 % cholic acid and 0.75 % cholesterol by Purina and water ad libitum for seven or eight days. Each test substance is then administered to a group of six rats in the supplemented diet for eight or twenty-one days, the test substance constituing 0.005-0.20 % of the diet. Body weight and food consumption data are recorded prior to diet administration, prior to test substance administration and at termination. Typical doses of the test substance are 4-200 mg/kg/day.

At termination, the rats are anesthetized, sacrificed by exsanguination, the blood is collected, kept on ice while clotting, centrifuged, and the serum is separated. An aliquot is adjusted to a density of 1.06 g/ml with sodium chloride solution and refrigerated for overnight storage. Remaining sera aliquots are refrigerated and frozen for storage.

HDL is isolated by micro-ultracentrifugation (mUC) or fast protein liquid chromatography (FPLC) techniques. The mUC method is as follows: 175 µl of the 1.06 g/ml density-adjusted blood serum is centrifuged at 42000 rpm in a Beckmann 42.2 Ti rotor at 20°C for 2.5 hours. 95 µl is fractionated from the top, leaving 80 µl in the bottom. FPLC fractionation is performed by Superose^{R} 6 (highly cross-linked beaded agarose having a

dry bead diameter of 20-40 μm) gel permeation chromatography using the method of Kieft et al., J.Lipid Res. 32 (1991) 859-866 modified by the technique described in France et al., Laboratory Robotics and Automation 2 (1990) 155-173. The column buffer is Tris buffered saline solution [i.e. 0.05 M Tris (2-amino-2-hydroxymethyl-1,3-propanediol) and 0.15 M sodium chloride in distilled deionized water (ddH_2O)] containing 0.01 % sodium azide. 200 μl of serum is injected and forty 0.5 ml fractions are collected.

Cholesterol is assayed on total, 42.2 Ti rotor bottoms and FPLC fractions using the Sigma Diagnostics kit for the enzymatic determination of cholesterol, Procedure No. 352, modified for use with ninety-six well microtiter plates. The reconstituted reagent (after addition of water) contains 300 U/l cholesterol oxidase, 100 U/l cholesterol esterase, 1000 U/l peroxidase (horseradish), 0.3 mmoles/l 4-aminoantipyrine and 30.0 mmoles/l p-hydroxybenzenesulfonate in a pH 6.5 buffer. This method uses cholesterol esterase to hydrolyze cholesterol esters to free cholesterol. Free cholesterol is oxidized to produce hydrogen peroxide that is used to form a quinoneimine dye. Since the reaction takes place quantitatively, the concentration of the dye, measured colorimetrically, is directly proportional to the cholesterol content of the sample. Calibrator, standard and sample may be diluted with saline if cholesterol concentrations cause readings outside the linear range. 20 μl of calibrator, standard or test sample is mixed with a 200 μl aliquot of reagent in a ninety-six well microtiter plate. Each mixture is incubated at 20°-25° for 25 minutes, and absorbance is determined by a colorimetric microtiter plate reader at 490, 492 or 500 nm.

Top cholesterol (i.e. LDL-cholesterol) is determined by subtraction. Quality of micro-ultracentrifugation separation is determined by Corning universal agarose gel electrophoresis with Fat Red 7B stain.

FPLC fraction Apo A-I content is assessed by the non-reducing SDS-PAGE method of France et al., J. Lipid Res. 30 (1989) 1997-2004. HDL-cholesterol is quantitated by assessing the cholesterol content of fractions containing Apo A-I and lacking immunoreactive Apo B protein.

Total triglycerides are assayed on blood serum using the Boehringer Mannheim Diagnostics Reagentset^{R} Triglycerides-GB kit (Cat. No. 877557) modified for microtiter plate assays as follows: The reagents are prepared according to the described method. 100 µl of Working Solution 1 and 20 µl of dilute blood serum [1:1 blood serum:saline (saline is 0.15 M sodium chloride in ddH_2O)] are added to each well of a ninety-six well microtiter plate, mixed and incubated at 20°-25° for at least 5 minutes. 100 µl of working solution 2 is added to each well, and the contents are mixed and incubated at 20°-25° for at least 5 minutes and read at 490, 492 or 500 nm. The total triglyceride content in mg/dl of blood serum is calculated by comparing the absorbance with the absorbance of known samples.

Other conventional methods may be used to assay the blood serum samples for HDL-cholesterol and total triglyceride content.

b) Test B: Apo A-I test:

The Apo A-I blood serum level of the test animals of Test A is determined by rat Apo A-I Enzyme-Linked Immunosorbent Assay (ELISA) as follows:

1. Production and purification of rabbit anti-rat Apo A-I antibody:

Rat Apo A-I is purified from pooled rat blood serum by sequential ultracentrifugation and isolation of rat high-density lipoproteins (HDL). Following delipidation, Apo A-I is resolved from other HDL proteins by gel filtration chromatography. Antisera are raised in three rabbits (Pocono Rabbit Farms, Candensis, PA) by serial subcutaneous injections of purified rat Apo A-I by conventional immunization protocols. After one year of repeated bleedings, antisera are pooled, aliquoted and frozen at -20°. The crude antisera are defrosted, applied to a cyanogen bromide-activated Sepharose^{R} Cl-4B (beaded agarose having a 60-140 µm dry bead diameter) column to which purified human HDL had been covalently linked, the column is washed with saline, and the purified antibody is eluted with 1 M pH 3 glycine solution. The glycine is removed by dialysis utilizing Tris Buffered Saline Solution to obtain a concentrated solution of purified rabbit anti-rat Apo A-I antibody.

2. Buffers, reagents and standards:

- Sodium carbonate adsorption buffer: 2.25 g of sodium carbonate, 4.40 g of sodium bicarbonate and 0.15 g of sodium azide are dissolved in 1.4 l of ddH_2O, the pH is adjusted to 9.6 with sodium hydroxide, and the buffer is diluted with ddH_2O to a total volume of 1.5 l;
- pH 8 0.5 M Tris: a solution of 60.55 g of Tris·HCl (2-amino-2-hydroxymethyl-1,3-propanediol·hydrochloride) in 1.0 l of ddH_2O is adjusted to pH 8.0 with 10 N sodium hydroxide solution;
- secondary antibody buffer: 200 ml of pH 8 0.5 M Tris is added to a solution of 40 g of bovine serum albumin (BSA) Cohn Fraction V in 1.8 l of ddH_2O, 0.2 g of sodium azide is added, and 18.0 g of sodium chloride is added;
- substrate buffer: 0.1 g of sodium azide is added with stirring to 105.1 g of diethanolamine and 500 ml of ddH_2O, 10 ml of 0.5 M magnesium chloride·hexahydrate solution is added, the pH is adjusted to 9.8 with 12 N hydrochloric acid, and the solution is diluted to a total volume of 1 l with ddH_2O;
- 30 x ELISA wash buffer: 525.96 g of sodium chloride, 76.68 g of Tris, 6 g of sodium azide and 30 ml of Tween^{R}20 [polyoxyethylene (20) sorbitan monolaurate] are combined, the pH is adjusted to 7.3 with 12 N hydrochloric acid, and the solution is diluted to a total volume of 2 l with ddH_2O;
- 1 x ELISA wash buffer: 1 volume of 30 x ELISA wash buffer is diluted with 29 volumes of ddH_2O;
- sample diluent buffer: 886.34 ml of secondary antibody buffer and 113.66 ml of Tween^{R}20 are mixed well to obtain an 11.366 % v/v Tween^{R}20 solution;
- standard diluent buffers: (A) 780 ml of secondary antibody buffer and 220 ml of Tween^{R}20 are mixed well to obtain a 22 % v/v Tween^{R}20 solution. (B) 890 ml of secondary antibody buffer and 110 ml of Tween^{R}20 are mixed well to obtain an 11 % v/v Tween^{R}20 solution;
- rat serum pool A: this source of rat blood serum is used to construct the standard curve for each assay. It is calibrated against purified Apo A-I and contains 45 ± 2.7 mg Apo A-I/dl blood serum;

- rat serum pool B: this is used as a low internal standard in each assay for monitoring assay drift and contains 40 mg Apo A-I/dl blood serum;
- rat serum pool C: this is derived from rats treated with reference compound (gemfibrozil), is used as a high internal standard for monitoring assay drift and contains 70 mg Apo A-I/dl blood serum.

3. Procedure:

Purified rat Apo A-I from a frozen stock containing 942 µg/ml is diluted in sodium carbonate adsorption buffer to a final concentration of 3.7 µg/ml. Into each well of several Nunc polystyrene ninety-six well microtiter plates is added 100 µl of this solution. Apo A-I is allowed to adsorb onto the plates for 48 hours at 4°. Plates are washed with 1 x ELISA wash buffer, and non-specific sites on the plate are blocked by an overnight incubation of secondary antibody buffer (200 µl/well) also in the cold. Assay plates are stored in this state until used (for up to three weeks).

On the day of the assay, ELISA platees (2 plates, 36 rat serum samples per plate) are washed four times in 1 x ELISA wash buffer. 100 µl of 1 x ELISA wash buffer is left in each well until the test sample is introduced. 10 µl of each rat blood serum sample to be analyzed is mixed with 290 µl of sample diluent buffer for a final dilution of 1:30 and a final Tween^{R}20 concentration of 11 %. Sextuplicate samples of internal standard rat serum pools B and C are treated identically.

2.0 ml of rat serum pool A is mixed with an equal volume of standard diluent buffer A to yield a dilution of 1:2 and a Tween^{R}20 concentration of 11 %. The sample is then serially diluted in standard diluent buffer B to yield dilutions of 1:8, 1:16, 1:32, 1:64 and 1:128.

Diluted standards and samples in non-ELISA microtiter plates are placed into a heated incubator at 52° for 2 hours. After cooling to 20°-25°, wash buffer is removed from ELISA test plates. 75 µl of standards and test samples are transferred to ELISA test plates. 75 µl of purified rabbit anti-rat Apo A-I antibody solution, diluted 1:75 in secondary antibody buffer, is then added to each well. Each plate is shaken briefly to assure mixing of antibody and sample, sealed and allowed to incubate for 18 hours at 20°-25°. After this incubation, each plate is washed in

1 x ELISA wash buffer four times. To each well is then added 100 µl of an alkaline phosphatase conjugated goat anti-rabbit IgG antibody, diluted 1:1000 in secondary antibody buffer. This is allowed to interact for 3 hours at 20°-25°. Each plate is washed again and aspirated dry, and to each well is added substrate buffer containing 1 mg/ml disodium p-nitrophenylphosphate, a chromogenic substrate which yields a color finversely proportional to the amount of Apo A-I in the sample. Each plate is monitored in an ELISA spectrophotometer for 1 to 3 hours. When wells which received no Apo A-I (maximum color) reach an optical density of 1.0 at 405 nm, each plate is read by the ELISA reader.

The standard curve is plotted as optical density versus log of the concentration. The unknowns are related to this curve, and the Apo A-I content is expressed in mg/dl.

The above tests A and B indicate that the compounds are active at a dosage in the range of from about 10 mg/kg to about 200 mg/kg per day.

The compounds are therefore indicated for use in raising the blood serum high density lipoprotein (HDL; HDL-cholesterol and apolipoprotein A-I) level and many of them also for lowering the blood serum total triglyceride level and thus in the treatment of atherosclerosis.

The precise dosage to be employed depends of course upon several factors including the host, the nature and the severity of the condition being treated, the mode of administration and the particular active substance employed. However, in general, satisfactory results are indicated to be obtained at daily dosages in the range of from about 400 mg to about 2000 mg, conveniently administered in divided doses two to four times a day.

The compounds may be administered by any conventional route, in particular enterally, preferably orally e.g. in the form of tablets or capsules, or parenterally e.g. in the form of sterile injectable solutions or suspensions.

The compounds of Examples 1, 2, 4, 95 and 96 are preferred, particularly those of Examples 1, 2 and 95, especially the compound of Example 2.

Thus e.g. the following activity has been determined:

Compound	Dose (mg/kg/day)	Duration of treatment (days)	HDL-cholesterol level increase (%)	Apo-I level increase (%)	Total triglycerides level decrease (%)
Example 1	60	8	225	65	45
Example 2	79	8	267	69	49
Gemfibrozil	50	8	100	50	35
Example 1	65	21	256	47	70
Example 2	73	21	323	102	44
Gemfibrozil	44	21	106	44	43

It is therefore indicated that, for this use, the compounds of Examples 1 and 2 may be administered at dosages lower than those conventionally employed with gemfibrozil by similar modes of administration, i.e. about 400-1000 mg/day orally.

Pharmaceutical compositions comprising a compound of formula I in free form or pharmaceutically acceptable salt form as appropriate together with at least one pharmaceutically acceptable carrier or diluent, may be manufactured in conventional manner. They may be e.g. in unit dosage form containing, for example, from about 100 mg to about 500 mg of active compound.

The invention thus concerns a method for the prophylactic or curative treatment of atherosclerosis comprising administering to a patient in need of such treatment a therapeutically effective amount of a compound of formula I in free form or pharmaceutically acceptable salt form as appropriate.

It further concerns a process for the preparation of a medicament against atherosclerosis which comprises mixing a compound of formula I in free form or salt form as appropriate with at least one pharmaceutically acceptable carrier or diluent.

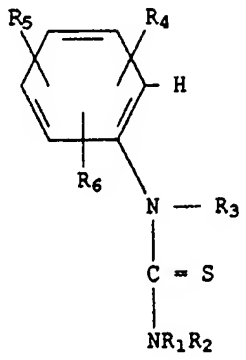
It further comprises the use of a compound of formula I in free form or salt form as appropriate for the manufacture of a medicament against atherosclerosis.

The invention also concerns a pharmaceutical composition comprising a compound of formula Ia in free form or pharmaceutically acceptable salt form as appropriate, together with at least one pharmaceutically acceptable carrier or diluent.

It further comprises a compound of formula Ia in free form or pharmaceutically acceptable salt form as appropriate, for use as a pharmaceutical, particularly for use in the prophylactic or curative treatment of atherosclerosis.

C L A I M S:

1. Use of a compound of formula I



I

wherein

either

R_1 is hydrogen; alkyl of 1 to 22 carbon atoms; cycloalkyl of 3 to 6 carbon atoms optionally mono- or independently disubstituted by alkyl of 1 or 2 carbon atoms; cycloalkylalkyl of 3 to 6 carbon atoms in the cycloalkyl and of 1 to 3 carbon atoms in the alkylene part or phenylalkyl of altogether 7 to 9 carbon atoms; hydroxyalkyl, alkoxyalkyl or mercaptoalkyl of 2 to 4 carbon atoms in the alkylene part, of 1 to 3 carbon atoms in the alkoxy part, and with the hydroxy, alkoxy or mercapto part attached in other than the 1-position; $-CR_7R_8R_9$ or $-CH_2CR_7R_8R_9$ wherein R_7 and R_8 either independently are hydrogen, methyl or ethyl or together are unbranched alkylene of 3 to 5 carbon atoms, and R_9 is carboxy, alkoxycarbonyl of altogether 2 to 4 carbon atoms or carbamoyl; alkenyl of 3 to 22 carbon atoms wherein the double bond is in other than the 1-position, or alkadienyl of 5 to 22 carbon atoms or alkatrienyl of 7 to 22 carbon atoms wherein each double bond is in other than the 1-position and no carbon atom is part of two double bonds, and

R_2 is hydrogen;

or

$-NR_1R_2$ is pyrrolidino, piperidino, hexamethyleneimino or morpholino, optionally mono-, di- or trisubstituted by alkyl of 1 to 4 carbon atoms or monosubstituted by hydroxymethyl or dialkylaminomethyl of independently 1 to 3 carbon atoms in the alkyl parts; piperazino substituted at the second nitrogen atom by alkyl of 1 to 4 carbon atoms, cycloalkyl of 5 or 6 carbon atoms, phenylalkyl of altogether 7 to 9 carbon atoms, phenyl, formyl or alkylcarbonyl of altogether 2 to 4 carbon atoms; 3-azaspiro[5.5]undec-3-yl; or 3,4-benzopiperidino;

R_3 is hydrogen or alkyl of 1 to 3 carbon atoms;

R_4 is hydrogen; alkyl of 1 to 4 carbon atoms; alkoxy of 1 to 3 carbon atoms; halogen of atomic number of from 9 to 35; trifluoromethyl; cyano; dialkylamino of independently 1 to 3 carbon atoms in the alkyl parts; nitro; phenyl; or benzyl;

R_5 is hydrogen; alkyl of 1 to 4 carbon atoms; alkoxy of 1 to 3 carbon atoms; halogen of atomic number 9 or 17; or trifluoromethyl; and

R_6 is hydrogen; alkyl of 1 to 3 carbon atoms; or alkoxy of 1 to 3 carbon atoms;

with the provisos that

i) when both R_4 and R_5 are trifluoromethyl, then they are not ortho to each other;

ii) when R_1 is hydroxyalkyl and each of R_4, R_5 and R_6 is selected from hydrogen and alkyl, then at least two of R_4, R_5 and R_6 are alkyl;

iii) when R_1 is alkyl and R_4 is phenyl, then R_5 and R_6 are other than alkoxy; and

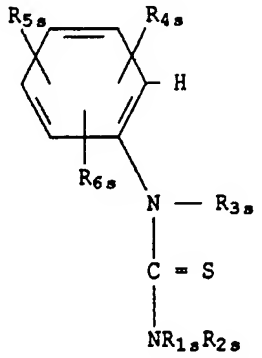
iv) when $-NR_1R_2$ is N'-alkylpiperazino and one of R_4 and R_5 is chloro, then both the other of R_4 and R_5, and R_6, are other than alkoxy;

in free form or salt form as appropriate,

for the manufacture of a medicament against atherosclerosis.

2. Use according to claim 1 wherein the compound of formula I is as defined in claim 1 with the further proviso that R_4 is other than benzyl and R_5 is other than alkyl of 4 carbon atoms.

3. Use according to claim 1 wherein the compound of formula I is a compound of formula Is



Is

wherein

either

R_{1s} is hydrogen; alkyl of 1 to 6 carbon atoms; cycloalkyl of 3 to 6 carbon atoms optionally monosubstituted by methyl; cyclopropylmethyl; phenylalkyl of 7 or 8 carbon atoms; hydroxyalkyl of 2 to 4 carbon atoms, methoxyethyl or mercaptoethyl with the hydroxy, methoxy or mercapto part attached in other than the 1-position; $-CR_{7s}R_{8s}R_{9s}$ or $-CH_2CR_{7s}R_{8s}R_{9s}$ wherein R_{7s} and R_{8s} either independently are hydrogen or methyl or together are unbranched alkylene of 3 to 5 carbon atoms, and R_{9s} is carboxy, methoxycarbonyl or carbamoyl; alkenyl of 3 to 18 carbon atoms wherein the double bond is in other than the 1-position, or alkadienyl of 5 to 18 carbon atoms or alkatrienyl of 7 to 18 carbon atoms wherein each double bond is in other than the 1-position and no carbon atom is part of two double bonds, and

R_{2s} is hydrogen;

or

$-NR_{1s}R_{2s}$ is pyrrolidino, piperidino, hexamethyleneimino or morpholino optionally mono- or disubstituted by methyl or ethyl or monosubstituted by hydroxymethyl or dimethylaminomethyl; piperazino substituted at the second nitrogen atom by alkyl of 1 to 3 carbon atoms, cyclohexyl, benzyl, 2-phenylethyl, phenyl, formyl or acetyl; 3-azaspiro[5.5]undec-3-yl; or 3,4-benzopiperidino;

R_{3s} is hydrogen or methyl;

R_{4s} is hydrogen; alkyl of 1 to 4 carbon atoms; methoxy; halogen of atomic number of from 9 to 35; trifluoromethyl; cyano; dimethylamino; nitro; phenyl; or benzyl;

R_{5s} is hydrogen; alkyl of 1 to 4 carbon atoms; methoxy; halogen of atomic number 9 or 17; or trifluoromethyl; and

R_{6s} is hydrogen; alkyl of 1 to 3 carbon atoms; or methoxy;

with the provisos that

i) when both R_{4s} and R_{5s} are trifluoromethyl, then they are not ortho to each other;

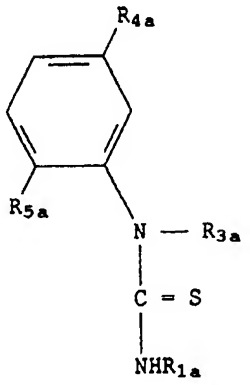
ii) when R_{1s} is hydroxyalkyl and each of R_{4s} and R_{5s} is selected from hydrogen and alkyl, then at least two of R_{4s}, R_{5s} and R_{6s} are alkyl;

iii) when R_{1s} is alkyl and R_{4s} is phenyl, then R_{5s} and R_{6s} are other than methoxy; and

iv) when $-NR_{1s}R_{2s}$ is N'-alkylpiperazino and one of R_{4s} and R_{5s} is chloro, then both the other of R_{4s} and R_{5s}, and R_{6s}, are other than methoxy;

in free form or salt form as appropriate.

4. A pharmaceutical composition comprising a compound of formula I as defined in claim 1 which is of formula Ia



Ia

wherein

R_{1a} is hydrogen; alkyl of 3 to 22 carbon atoms; cycloalkyl of 3 to 6 carbon atoms optionally mono- or independently disubstituted by alkyl of 1 or 2 carbon atoms; cycloalkylalkyl of 3 to 6 carbon atoms in the cycloalkyl and of 1 to 3 carbon atoms in the alkylene part; alkoxyalkyl or mercaptoalkyl of 2 to 4 carbon atoms in the alkylene part, of 1 to 3 carbon atoms in the alkoxy part, and with the alkoxy or mercapto part attached in other than the 1-position; $-CR_{7a}R_{8a}R_{9a}$ or $-CH_2CR_{7a}R_{8a}R_{9a}$ wherein R_{7a} and R_{8a} either independently are hydrogen, methyl or ethyl or together are unbranched alkylene of 3 to 5 carbon atoms, and R_{9a} is alkoxycarbonyl of altogether 2 to 4 carbon atoms or carbamoyl; alkenyl of 4 to 22 carbon atoms wherein the double bond is in other than the 1-position, or alkadienyl of 5 to 22 carbon atoms or alkatrienyl of 7 to 22 carbon atoms wherein each double bond is in other than the 1-position and no carbon atom is part of two double bonds;

R_{3a} is hydrogen or alkyl of 1 to 3 carbon atoms;

one of R_{4a} and R_{5a} is alkyl of 1 to 4 carbon atoms; alkoxy of 1 to 3 carbon atoms; halogen of atomic number of from 9 to 35; trifluoromethyl; cyano; dialkylamino of independently 1 to 3 carbon atoms in the alkyl parts; nitro; phenyl; or benzyl;

the other of R_{4a} and R_{5a} is alkyl of 1 to 4 carbon atoms; alkoxy of 1 to 3 carbon atoms; or trifluoromethyl;

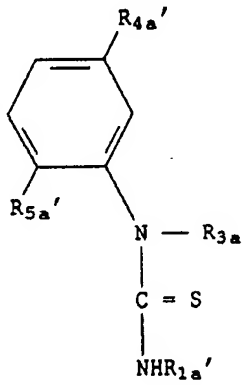
with the provisos that

i) when both R_{4a} and R_{5a} are trifluoromethyl,
then they are not ortho to each other; and

ii) when R_{1a} is alkyl and one of R_{4a} and R_{5a} is phenyl,
then the other of R_{4a} and R_{5a} is other than alkoxy;

in free form or salt form as appropriate,
together with a pharmaceutically acceptable carrier or diluent.

5. A pharmaceutical composition according to claim 4 wherein the compound of formula Ia is of formula Ia'



Ia'

wherein

R_{1a}' is propyl or isopropyl or alkyl of 4 to 22 carbon atoms branched at a position other than at the carbon atom attached to the nitrogen atom; cycloalkyl of 3 to 6 carbon atoms optionally mono- or independently disubstituted by alkyl of 1 or 2 carbon atoms; cycloalkylalkyl of 3 to 6 carbon atoms in the cycloalkyl and of 1 to 3 carbon atoms in the alkylene part; alkoxyalkyl or mercaptoalkyl of 2 to 4 carbon atoms in the alkylene part, of 1 to 3 carbon atoms in the alkoxy part, and with the alkoxy or mercapto part attached in other than the 1-position; $-CR_{7a}R_{8a}R_{9a}$ or $-CH_2CR_{7a}R_{8a}R_{9a}$ wherein R_{7a}, R_{8a} and R_{9a} are as defined above; alkenyl of 4 to 22 carbon atoms wherein the double bond is in other than the 1-position, or alkadienyl of 5 to 22 carbon atoms or alkatrienyl of 7 to 22 carbon atoms wherein each double bond is in

other than the 1-position and no carbon atom is part of two double bonds;

R_{3a} is as defined in claim 4;

one of R_{4a}' and R_{5a}' is methyl; alkoxy of 1 to 3 carbon atoms; halogen of atomic number of from 9 to 35; trifluoromethyl; cyano; dialkylamino of independently 1 to 3 carbon atoms in the alkyl parts; nitro; phenyl; or benzyl;

the other of R_{4a}' and R_{5a}' is methyl; alkoxy of 1 to 3 carbon atoms; or trifluoromethyl;

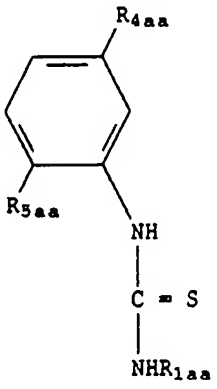
with the provisos that

i) when both R_{4a}' and R_{5a}' are trifluoromethyl, then they are not ortho to each other;

ii) when R_{1a}' is alkyl and one of R_{4a}' and R_{5a}' is phenyl, then the other of R_{4a}' and R_{5a}' is other than alkoxy; and

iii) R_{4a}' and R_{5a}' are other than both alkoxy.

6. A compound of formula I as defined in claim 1 which is of formula Iaa



Iaa

wherein

R_{1aa} is propyl, isopropyl or 2-methylpropyl;

R_{4aa} is halogen of atomic number of from 9 to 35; and

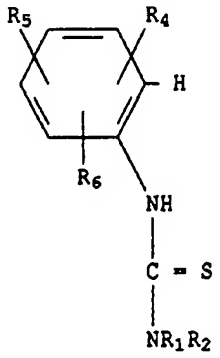
R_{5aa} is alkyl of 1 to 4 carbon atoms.

7. The compound of formula I according to claim 6 which is N-(5-chloro-2-methylphenyl)-N'-propylthiourea.

8. The compound of formula I according to claim 6 which is N-(5-chloro-2-methylphenyl)-N'-2-methylpropylthiourea.

9. The compound of formula Iaa according to claim 6 wherein R_{1aa}, R_{4aa} and R_{5aa} are, respectively, either
- $CH(CH_3)_2$, 5-Cl and 2-CH_3, or
- $CH_2CH_2CH_3$, 5-Cl and 2-CH_2CH_3, or
- $CH_2CH_2CH_3$, 5-F and 2-CH_3, or
- $CH_2CH_2CH_3$, 5-Br and 2-CH_3.

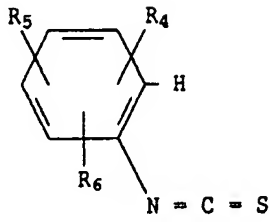
10. A process for the preparation of a compound of formula I as defined in claim 1 which comprises
a) for the preparation of a compound of formula I'



I'

wherein the substituents are as defined in claim 1,

reacting a corresponding compound of formula II



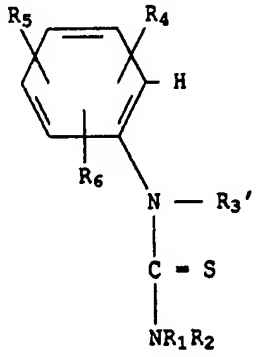
II

with a corresponding compound of formula III

$$R_1R_2NH \qquad \text{III}$$

or

b) for the preparation of a compound of formula I"

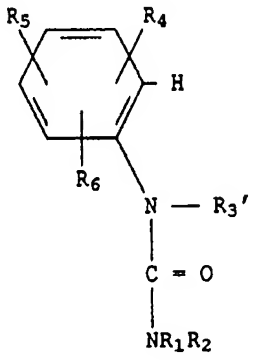


I"

wherein R_3' is alkyl of 1 to 3 carbon atoms and

the remaining substituents are as defined in claim 1,

appropriately converting the oxo moiety to a thiono moiety in a corresponding compound of formula IV



IV

and recovering the resultant compound of formula I in free form or salt form as appropriate.